REMARKS

Applicants have merely deleted the BRIEF DESCRIPTION OF THE DRAWINGS at pages 61 and 62 and inserted each of Figures 1A to Figure 7 in the specific example where the figures are discussed. Thus, Figure 1b was moved to Example 12 on page 50. Figure 1a and Figure 2 were moved to Example 16 on page 53. Figures 3 and 4 were moved respectively to Examples 20 and 21 on page 55. Figure 5 was moved to Example 22 on page 56. Figure 6 was moved to Example 24 on page 57. Figure 7 was moved to Example 28 on page 60.

Please note that Figure 1b is not referred to in Example 12 but the BRIEF DESCRIPTION OF THE DRAWINGS at page 61 identifies that figure as representing "an HPLC separation of (+)-catechin-(4α,8)-(-)-epicatechin prepared by Example 12" and that the title of Example 12 (see page 50) is "Preparation of (+)-Catechin (4α→8)-(-)-epicatechin."

Copies of the relevant pages are attached as Exhibit 2 and copies of the figures are attached as Exhibit 3.

Entry of this Amendment is respectfully requested. No new matter is presented. If requested, a substitute specification will be provided.

This procedure was used to correct prior application Serial No. 10/212,973 filed August 6, 2002.

If the amendment is not entered, the drawings will be cancelled as they are not required for an understanding of how the procyanidins are synthetically prepared.

Respectfully submitted,

Date: August ~~17~~ 18, 2004

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APPLICATION NUMBER	FILING OR 371 (c) DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NUMBER
10/798,131	03/11/2004	Leo J. Romanczyk JR.	5677-211

Margaret B. Kelley
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CONFIRMATION NO. 5849

FORMALITIES LETTER

OC000000013287255

Date Mailed: 07/21/2004

NOTICE TO FILE CORRECTED APPLICATION PAPERS

Filing Date Granted

An application number and filing date have been accorded to this application. The application is informal since it does not comply with the regulations for the reason(s) indicated below. Applicant is given TWO MONTHS from the date of this Notice within which to correct the informalities indicated below. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

The required item(s) identified below must be timely submitted to avoid abandonment:

- Replacement drawings in compliance with 37 CFR 1.84 and 37 CFR 1.121 are required. The drawings submitted are not acceptable because:
  - The drawings have a line quality that is too light to be reproduced (weight of all lines and letters must be heavy enough to permit adequate reproduction) or text that is illegible (reference characters, sheet numbers, and view numbers must be plain and legible) see 37 CFR 1.84(l) and (p)(1)); See Figure(s) 1a, 1b.

Replies should be mailed to: Mail Stop Missing Parts
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RASANTD

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Initial Patent Examination Division (703) 308-1202

PART 1 - ATTORNEY/APPLICANT COPY

CH_2Ph), 4.29 (1H, dd, J=8.3, 8.3 Hz, A-4), 3.80 (IH, m, H-3), 2.71 (IH, d, J=16.6 Hz, B-4), 2.53 (1H, dd, J=4.4, 16.6 Hz, B-4); ^{13}C NMR δ156.5, 156.0, 154.6, 154.0, 152.5, 151.6, 151.4, 151.2, 150.6, 147.0, 146.7, 141.7, 141.6, 141.5, 139.8, 134.8, 134.4, 134.2, 133.9, 129.3, 128.7, 126.2, 126.1, 126.0, 125.8, 125.5, 125.4, 125.2, 125.1, 125.0, 124.9, 119.8, 115.0, 112.6, 111.2, 106.1, 104.5, 96.5, 94.9, 93.0, 92.8, 79.9, 70.5, 69.1, 69.0, 67.8, 67.7, 63.9, 35.0, 25.5; IR (KBr, cm^{-1}) 3418, 3057, 3034, 2918, 1609, 1510, 1446, 1371, 1260, 1202, 1097, 806, 731, 696; MS (FAB, *m/z*) 939.6 $(M + H)^+$, 649.1, 607.0, 559.0, 459.8.

Example 12

Preparation of (+) – Catechin (4α→8)-(-)-epicatechin

Tetra-*O*-benzyl-(+)-catechin-(4α→8)-(-)-epicatechin prepared in Example 11 (50 mg) was dissolved in methanol (10 mL) and degassed by blowing argon for 10 min. 30% Palladium-charcoal (30 mg) was added and hydrogenolysis conducted at 45 psi for 3 hours. The solution was filtered through Celite which was washed with methanol. The combined filtrate and washings were evaporated and the residue was dissolved in water, then lyophilized to provide a quantitative yield of the dimer as an off-white solid. For the NMR spectrum the Hs comprising the upper monomer of the dimer are designated A and the Hs comprising the lower monomer of the dimer are designated B. ^1H NMR ($CDCl_3$:d_4-methanol, 9:1) δ_H 7.21 (1H, bs, A-2'), 7.04 (1H, bs, B-2'), 6.95-6.75 (2H, m, A-5', B-5'), 6.62 (1H, m, A-6'), 6.45 (1H, m, B-6'), 6.20 (1H, m, B-6), 6.05 (1H, m, B-6), 5.89 (2H, m, A-6, A-8), 4.98 (1H, m, B-2,), 4.85 (1H, m, B-2), 4.42-4.25 (3H, m, A-4, A-3, A-2), 3.05-2.62 (2H, m, B-4).

Example 13

Preparation of 3-Acetyl-tetra-*O*-benzyl-(+)-catechin-(4α→8)-pentaacetyl-(-)-epicatechin

Tetra-*O*-benzyl-(+)-catechin-(4α→8)-epicatechin prepared in Example 11 was acetylated with acetic anhydride in pyridine. 120 mg of tetra *O*-benzyl-(+)-catechin (4α→8)-(-)-epicatechin was dissolved in 2 mL of dry pyridine and 500 μL of acetic anhydride added. The reaction mixture was stirred under

Example 16

Preparation of (-)-Epicatechin-(4β→8)-(-)-epicatechin

Tetra-*O*-(-)epicatechin (4β→8)-(-)-epicatechin prepared in Example 15 (40 mg, 0.043 mmol) was dissolved in 8 mL methanol and degassed by blowing argon for 10 min. To the solution, 25 mg of 30% palladium-charcoal was added and the mixture hydrogenolyzed at 45 psi for 3 hours. The solution was filtered through Celite followed by washing with 25 mL methanol. The combined filtrate and washing were evaporated and the residue dissolved in water. Lyophilization provided 23 mg of an off-white powder. HPLC analysis (Figure 1A) revealed the presence of 18% monomer, 45% dimer, 25% trimer and 8% tetramer. The ^{1}H NMR spectrum is shown in Figure 2.

Example 17

Preparation of Tetra-*O*-benzyl-(+)-catechin-(4α→8)-(+)-catechin

4β-Acetoxy tetra-*O*-benzyl-(+)-catechin prepared by Example 6 (70 mg. 0.1 mmol), (+)-catechin (145 mg, 5 eq) and LiBr (44 mg, 5 eq) were dissolved in a mixture of THF and methylene chloride (3 mL each) and the solution refluxed for 24 hours under argon. The solution was partitioned between ethyl acetate and water (25 mL each) and the organic phase dried over Na_2SO_4. Following evaporation, the residue was resuspended in ethyl acetate (25 mL) and filtered to remove most of the unreacted (+)-catechin. After evaporation, the residue was subjected to silica gel chromatography where elution with methylene chloride:ethyl acetate (1:1, v/v) provided an off-white powder (81 mg, 68%) after evaporation. ^{1}H NMR ($CDCl_3$:d_4-methanol, 9:1) δ_H 7.39-7.06 (20H, m), 6.84-6.68 (5H, m), 6.47 (1H, d, J=7.9 Hz), 6.32-5.98 (4H, m), 5.00-4.33 (11H, m), 3.58 (1H, m), 2.98 (1H, m), 2.35 (1H, m); IR (KBr, cm^{-1}) 3441, 3057, 3034, 2918, 1609, 1542, 1510, 1371, 1266, 1097, 812, 737, 696; MS (APCI, *m/z*) 938 (M-H), 920, 848, 816, 696, 607, 558.

monomer of the dimer are designated B. ^{1}H NMR ($CDCl_3$ d_4-methanol, 9:1) δ_H 7.41-7.13 (40H, m, Ar-H), 6.97-6.79 (6H, complex, A-2', A-5', A-6', B-2', B-5', B-6'), 6.22 (1H, s, B-6), 6.20, 6.12 (2x1H, 2xd, J=2.4 Hz, A-6, A-8), 5.18-4.51 (19H, CH_2Ph, A-2, A-4, B-2), 4.28 (1H, m, A-3), 3.85 (1H, m, B-3), 2.95 (1H, d, J=16 Hz, B-4), 2.60 (1H, dd, J=5, 16 Hz, B-4).

Example 20

Preparation of Tetra-*O*-benzyl-(+) catechin -(4α→8)-3-acetyl-(+)-catechin-(4α→8)-pentaacetyl (-)-epicatechin

To a solution of 3-acetyl -(+)-catechin-(4α→8)-pentaacetyl-(-)-epicatechin prepared by Example 14 (100 mg, 0.068 mmol) and 4β acetoxy tetra-*O*-benzyl-(+)-catechin prepared by Example 6 (334 mg, 2 eq) in THF (7 mL) and methylene chloride (7 mL), 161 mg of LiI was added. The solution was refluxed for 24 hours, followed by partition between ethyl acetate and water (25 mL each). The organic phase was dried over Na_2SO_4, filtered and the solvent evaporated. The residue was subjected to silica gel chromatography where elution with ethyl acetate-methylene chloride (1:1, v/v) provided a brownish white solid (100 mg, 28%) after the evaporation of the solvent. The resulting ^{1}H NMR is shown in Figure 3. MS (FAB, *m/z*) 1482 $(M+H)^+$, 1148, 1042, 962, 920, 650.

Example 21

Preparation of Tetra-*O*-benzyl-(+)-catechin -(4α→8) -pentaacetyl-(+)-catechin-(4α→8)-pentaacetyl (-)-epicatechin

Tetra-*O*-benzyl-(+) catechin-(4α→8)-3-acetyl-(+)-catechin-(4α→8)-pentaacetyl (-)-epicatechin prepared by Example 20 (100 mg, 0.068 mmol) was stirred in dry pyridine (2 mL) and acetic anhydride (1 mL) under argon for 24 hours. The solution was then partitioned between 1N HCl and ethyl acetate (25 mL each), the organic layer was washed with 5% $NaHCO_3$, saturated NaCl and dried over $MgSO_4$. Evaporation of the solvent provided an oily residue which was subjected to silica gel chromatography where elution with 10% ethyl acetate in methylene chloride provided a white powder (70 mg, 61%) after evaporation of the solvent. The resulting ^{1}H NMR is shown in Figure 4.

Example 22

<u>Preparation of 3 Acetyl-(+)-catechin -(4α→8) -pentaacetyl-(+)-catechin-(4α→8)-pentaacetyl (-)-epicatechin</u>

Tetra-*O*-benzyl- (+)-catechin-(4α→8)-pentaacetyl-(+)-catechin-(4α→8)-pentaacetyl (-)-epicatechin prepared in Example 21 (50 mg) was dissolved in degassed ethyl acetate-methanol (3 mL each) and hydrogenolysed for 4 hours in the presence of 30% palladium-charcoal (30 mg) at 45 psi. Removal of the catalyst via filtration through Celite and evaporation provided the title compound as a pale brown powder (35 mg, 91%). The resulting ^{1}H NMR is show in Figure 5.

Example 23

<u>Tetra-*O*-benzyl-(+)-catechin-(4α→8)-3-acetyl-(+)-catechin -(4α→8)-pentaacetyl-(+)-catechin-(4α→8)-pentaacetyl (-)-epicatechin</u>

To a solution of 3-acetyl-(+)-catechin-(4α→8)-pentaacetyl-(+)-catechin-(4α→8)-pentaacetyl (-)-epicatechin prepared by Example 22 (30 mg, 0.0226 mmol) and 4ß-acetoxy tetra-*O*-benzyl-(+)-catechin prepared by Example 6 (31 mg, 2 eq) in THF and methylene chloride (2 mL each), LiI (16 mg, 5 eq) was added and the solution refluxed for 24 hours. The solution was partitioned between ethyl acetate and water (25 mL each) and the organic layer dried over $MgSO_4$, filtered and the solvent evaporated. The residue was subjected to silica gel chromatography where elution with 10% methanol in methylene chloride provided a brownish-white solid (20 mg, 45%) after evaporation of the solvent. MS (FAB, *m/z*) 1978 $(M + H)^+$, 1934 $(M^+ - COCH_3)$, 1571 $(M^+ - COCH_3, -3xCH_2Ph)$, 1646, 1430, 1373, 1330, 1269.

Example 24

<u>Preparation of Tetra-*O*-benzyl-(+)-catechin-(4α→8) -(-)-epicatechin-(6→4α)-tetra-*O*-benzyl-(+)-catechin</u>

To a solution of tetra-*O*-benzyl-(+)-catechin-(4α→8)-(-)-epicatechin prepared by Example 11 (69 mg, 0.074 mmol) and 4ß-acetoxy tetra-*O*-benzyl-(+)-catechin prepared by Example 6 (51 mg, 0.074 mmol) in methylene chloride and THF (5 mL each), LiBr (65 mg, 10eq) was added and the

mixture refluxed for 24 hours. The solution was partitioned between ethyl acetate and water (25 mL each) and the organic layer dried over $MgSO_4$. The solvent was evaporated and the residue subjected to silica gel chromatography where elution with ethyl acetate-methylene chloride (1:1, v/v) provided a white powder (35 mg, 30%) after evaporation of the solvent. The resulting ^{1}H NMR is shown in Figure 6. MS (FAB, *m/z*) 1588 $(M + H)^+$, 1255, 772, 648, 607, 560.

Example 25

<u>Preparation of 8-Bromo tetra-*O*-benzyl-(-)-epicatechin</u>

To a solution of tetra-*O*-benzyl-(-)-epicatechin (Example 2) (65 mg, 0.1 mmol) in methylene chloride (2 mL), N-bromosuccinimide (18 mg, 0.1 mmol) was added and the solution stirred under argon for 10 min. The mixture was filtered through silica gel followed by elution with 20 mL ethyl acetate:methylene chloride (1:1, v/v). The combined filtrate and eluant were evaporated. The residue was subjected to silica gel chromatography where elution with methylene chloride provided the title compound as shiny pinkish-white crystals (66 mg, 90%) after evaporation of the solvent. ^{1}H NMR ($CDCl_3$) δ_H, 7.45-7.21 (21 H, m, Ar-H), 7.01 (1H, dd, J=1.4 8.3 Hz, H-6'), 6.96 (1H, d, J=8.3 Hz, H-5'), 6.23 (2H, s, H-6), 5.38 (1H, m, H-3), 5.21, 5.18, 5.10, 4.97 (4x2H, 4xs, 4xCH_2), 5.01 (1H, s, H-2), 4.3 (1H, m, H-3), 3.03 (1H, dd, J=1.9, 17.4 Hz, H-4), 2.89 (1H, dd, J=4., 17.4 Hz, H-4), 1.55 (1H, d, J=4.8 Hz, OH).

Example 26

<u>Preparation of 8-Bromo pentabenzyl-(-)-epicatechin</u>

To a solution of pentabenzyl-(-)-epicatechin (55 mg, 0.074 mmol) in methylene chloride (2 mL) at 0°C, N-bromosuccinamide (14 mg, 1eq) was added and the solution stirred at r.t. for 30 min. The solution was passed through a 25 mm dia. column of silica gel (7 gm) which was eluted with methylene chloride (30 mL). The combined filtrate and eluant were evaporated to provide the title compound as a white foam (50 mg, 82.5%) after evaporation of the solvent. ^{1}H NMR ($CDCl_3$) δ_H, 7.43-6.90 (28H, m, Ar-H), 6.21 (1H, s, H-6), 5.17 (2H, s, CH_2), 5.09 (5H, s, 2xCH_2, H-2), 4.96 (2H, s, CH_2), 4.37,

The assignment of the correct absolute configuration was tested by calculation of the Flack 'x' parameter. This parameter was indistinguishable from zero, indicating the correct configuration was assigned. A test refinement of the inverted configuration resulted in a Flack 'x' parameter value of 0.95(5) and a significant increase in the R factors, both indicating that the assignment was correct. The final model for 8-bromo tetra-*O*-benzyl-(-)-epicatechin is shown in Figure 7.

Example 29

Preparation of (8↔8), (8↔6), and (6↔6) Linked Procyanidin Oligomers

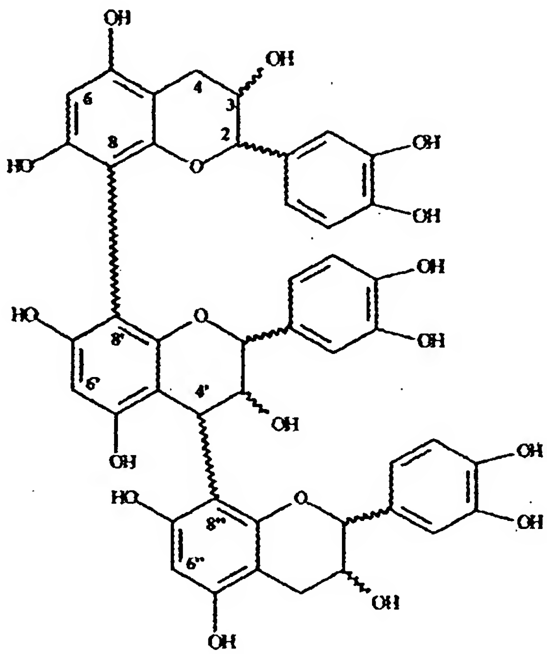
The steps described in this invention can be extended to provide procyanidin oligomers comprising (8↔8), (8↔6), (6↔6) interflavan linkages. These compounds are obtained from 6-bromo- and/or 8-bromo-(monomer) intermediates. Coupling of these brominated monomers with organotin derivatives by a Stille reaction in the presence of a $palladium_{(0)}$ catalyst leads to the desired oligomeric linkage. (Stille, J.K., *Agnew, Chem. Internal. Ed. Engl.*, 25, 508-524 (1986)).

For instance, 8-bromo pentabenzyl-(-)-epicatechin prepared by Example 26 is reacted with hexaabutyl distannane to provide the alkyl stannane of pentabenzyl (-)-epicatechin. Coupling of this stannnane with another 8-bromo pentabenzyl (-)-epicatechin in the presence of tetrakis (triphenyl phosphine) $palladium_{(0)}$ in benzene provides the deca benzyl (-) epicatechin dimer with a (8↔8) linkage. Deprotecting with H_2/Pd provides the (-)-epicatechin-(8↔8)-(-)-epicatechin in free phenolic form.

Similarly, procyanidin oligomers comprising (8↔6) or (6↔6) linkages can be synthesized using the appropriate 6-bromo- or 8-bromo-(monomer) derivatives. Further, coupling of 8-bromo- or 6-bromo- dimers, trimers and higher oligomers can provide "even" numbered procyanidin oligomers comprising (8↔8), (8↔6), and (6↔6) linkages.

Still further, coupling of blocked monomers used to prepare (4→6) linked oligomers as described in the invention can be used in the Stille reaction to provide novel procyanidin oligomers comprising combinations of the (4→6) and (4→8) linked oligomers with (8↔8), (8↔6), and (6↔6)

linkages. By way of example, the following structure illustrates and (8↔8) and (4↔8) linked procyanidin trimer.



BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 A	Represents an HPLC separation of (-)-epicatechin-(4β→8)-(-) epicatechin prepared by Example 16
Figure 1B	Represents an HPLC separation of (+)-catechin-(4α→8)-(-)-epicatechin prepared by Example 12
Figure 2	Represents the ¹H NMR spectrum of (-)-epicatechin-(4β→8)-(-)epicatechin prepared by Example 16
Figure 3	Represents the ¹H NMR spectrum of tetra-*O*-benzyl-(-)-catechin-(4α→8)-pentaacetyl (-)-epicatechin prepared by Example 20
Figure 4	Represents the ¹H NMR spectrum of tetra-*O*-benzyl (+)-catechin-(4α→8)-pentaacetyl (+)-catechin-(4α→8)-(-)-epicatechin prepared by Example 21

NYB 1035783.1

Figure 5	Represents the ^{1}H NMR spectrum of tetra-*O*-benzyl (+)-catechin-(4α→8)-pentaacetyl (+)-catechin-(4α→8)-pentaacetyl (-)-epicatechin prepared by Example 22
Figure 6	Represents the ^{1}H NMR spectrum of tetra-*O*-benzyl (+)-catechin-(4α→8)-(-)-epicatechin-(6→4α) tetra-*O*-benzyl (+)-catechin prepared by Example 24
Figure 7	Represents a X-ray model for 8-bromo tetra-*O*-benzyl (-)-epicatechin

Figure 1b

Johns Hopkins Dimer AB-1-97 (0.22mg/0.1mL) for Lee;nega
tive mode; with column; NH4OH @ 0.04 ml/min (~0.75 M);
fragmentor 75; Vcap 3000; Scan after capillary clean

Catechin - Epicatechin

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		Inj Volume :	20 µl
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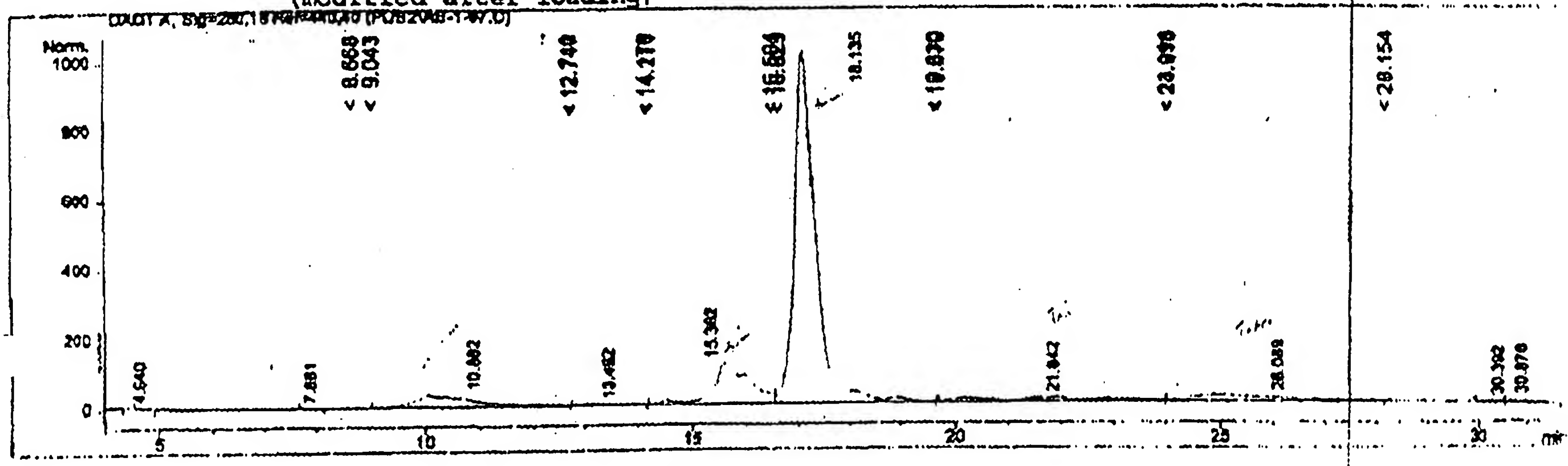

Area Percent Report

Sorted By	:	Signal
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Dilution	:	1.0000

Figure 1a

Johns Hopkins Dimer AB-1-98 (0.21mg/0.1mL) for Lee;negative mode; with column; NH4OH @ 0.04 ml/min (~0.75 M); fragmentor 75; Vcap 3000; Scan after capillary clean

Epi Catechin - Epicatechin

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Acq. Operator	: Sherri Lazarus	Inj Volume :	20 µl

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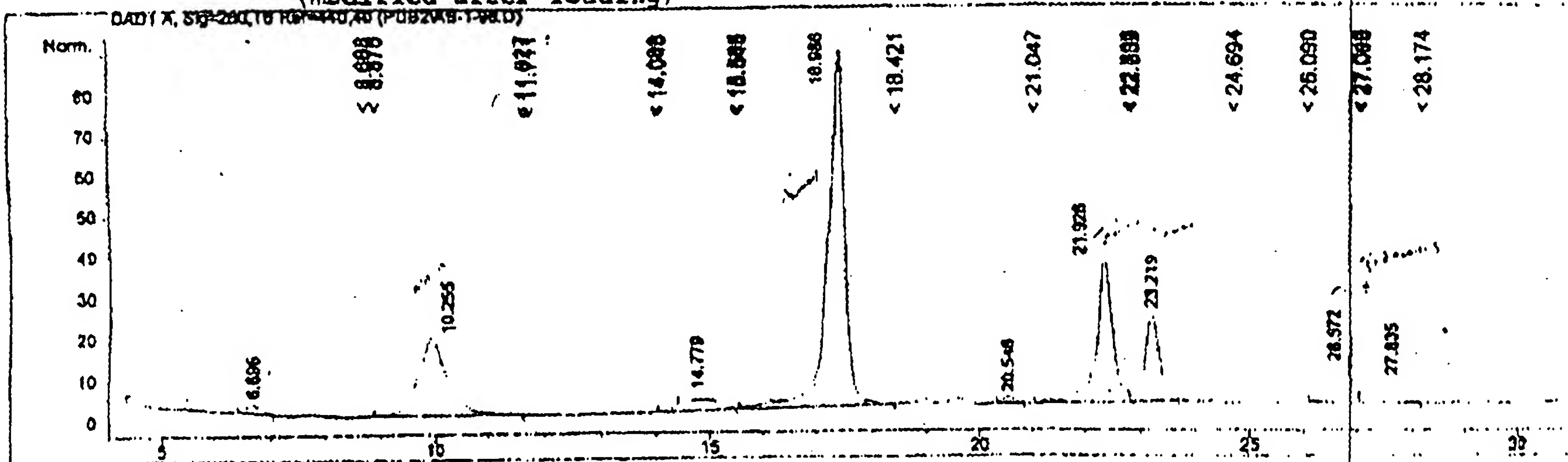

Area Percent Report

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Figure 2

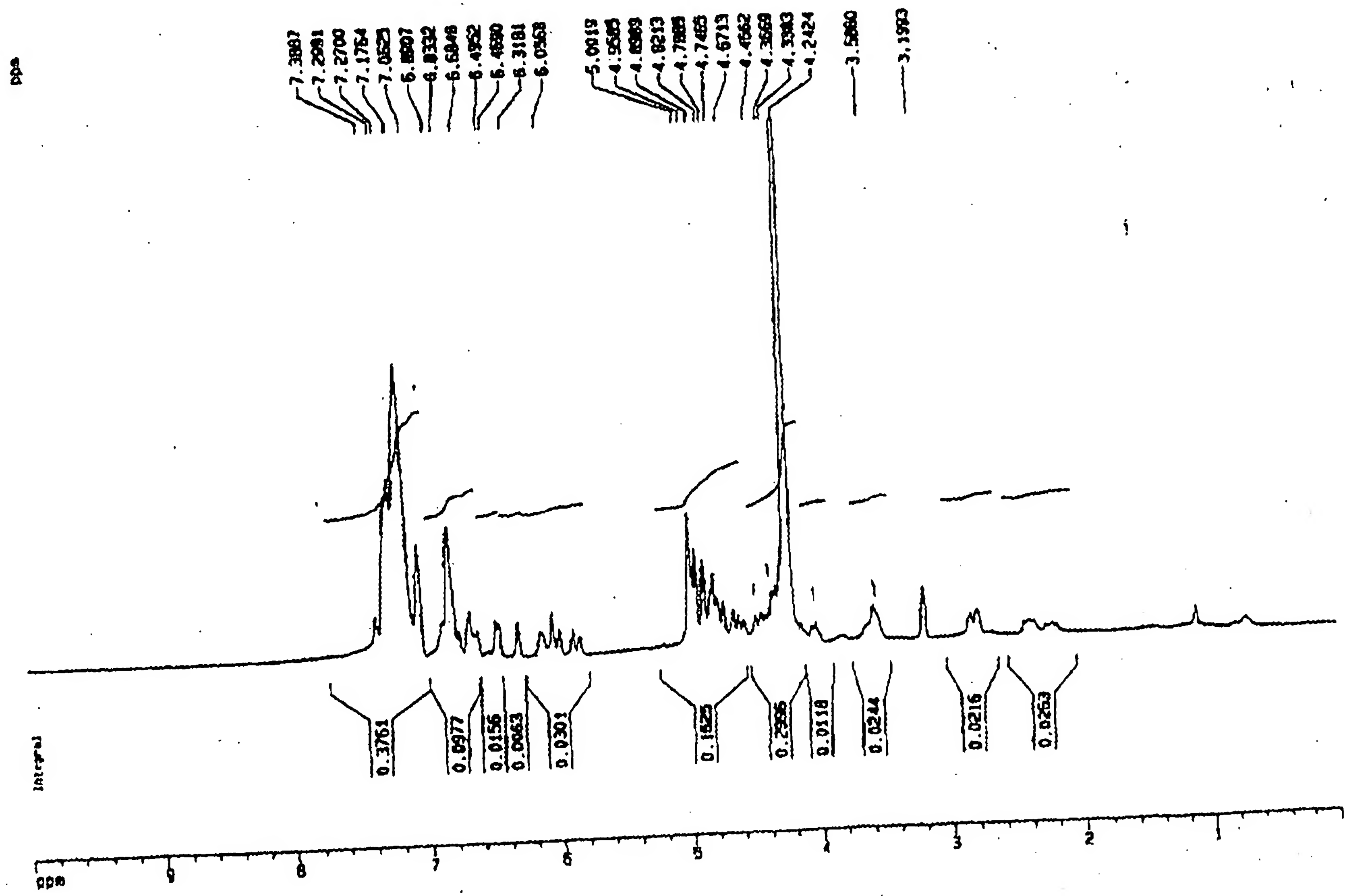
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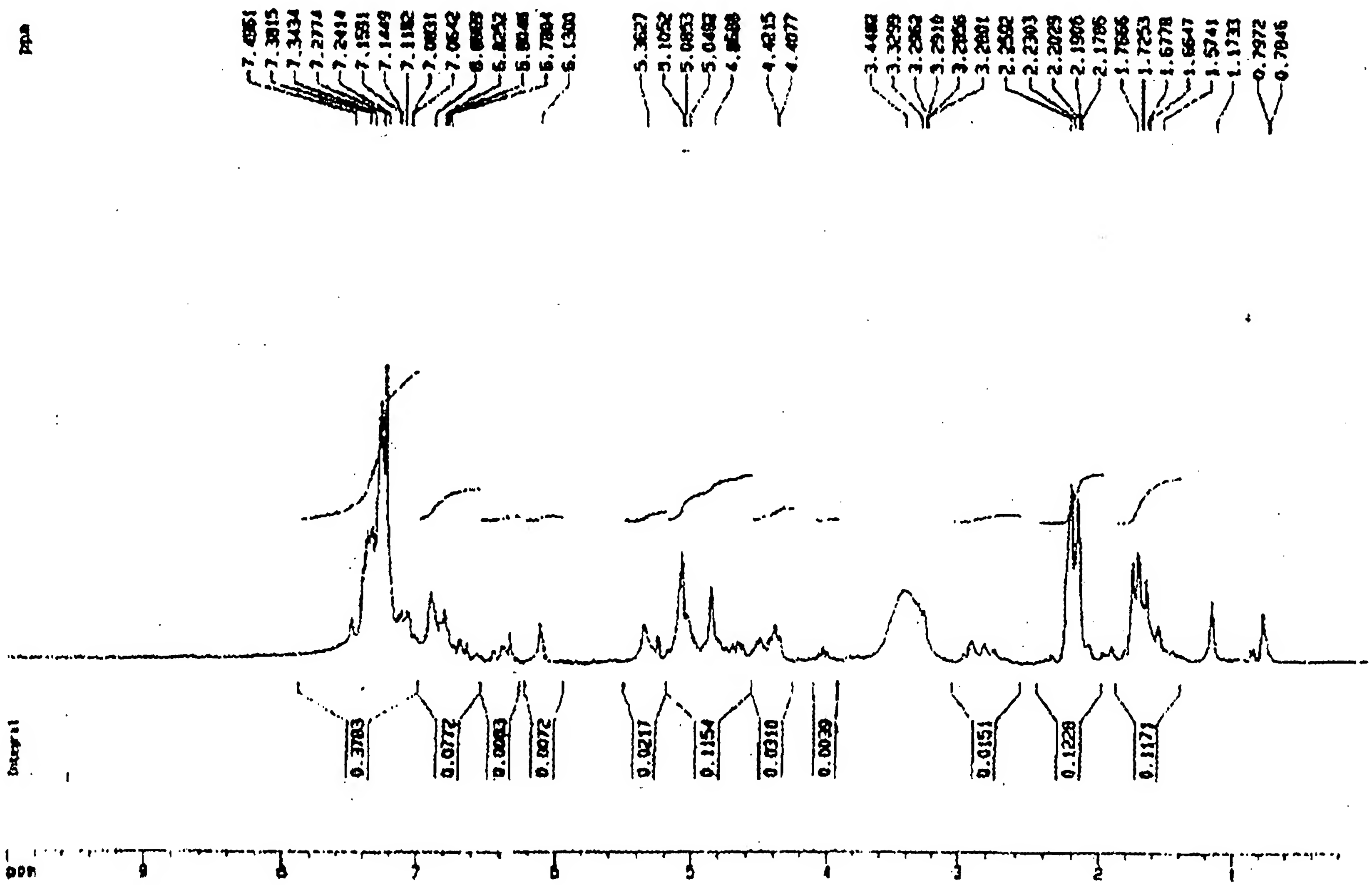
Figure 3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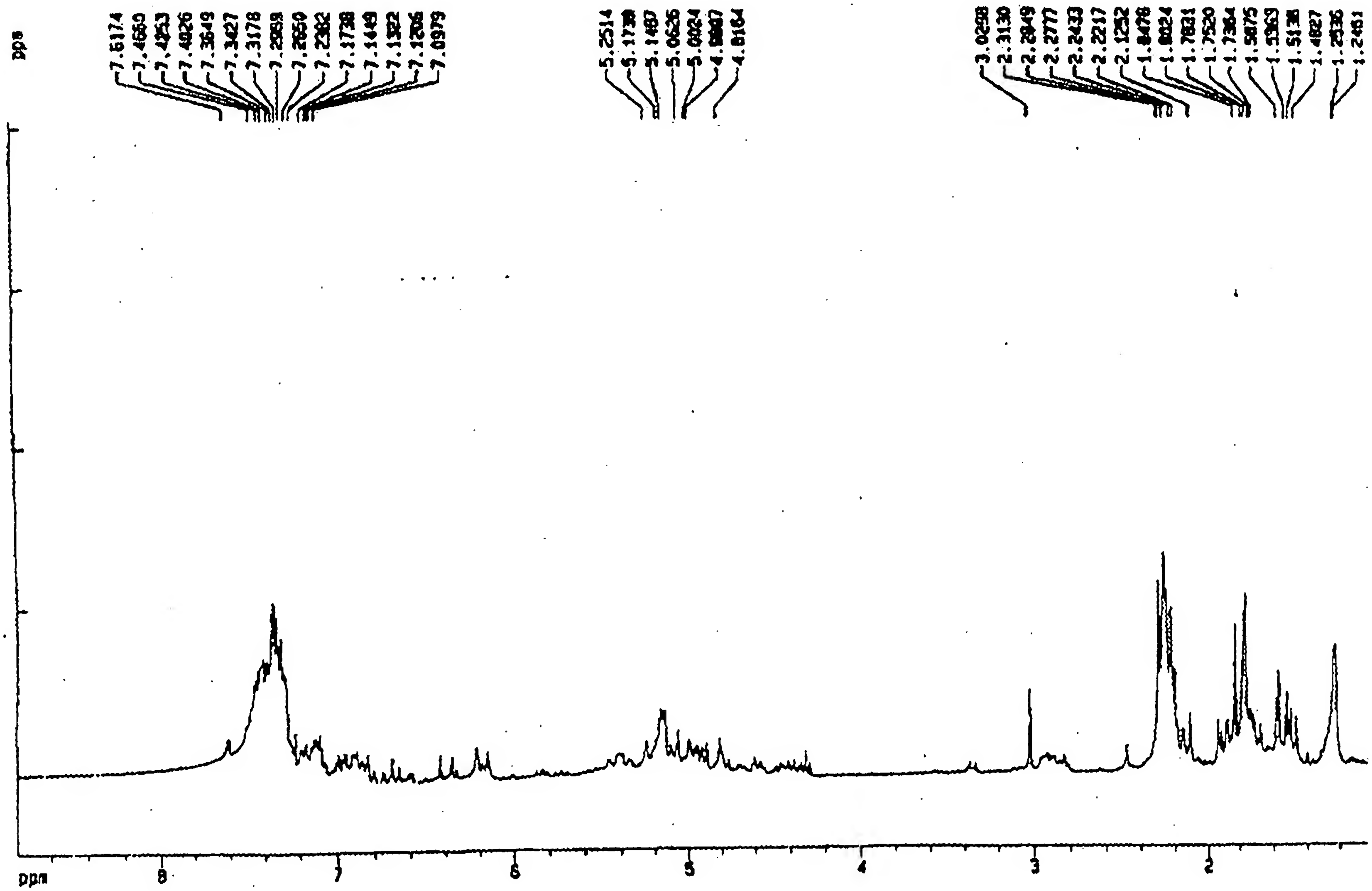
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Figure 5

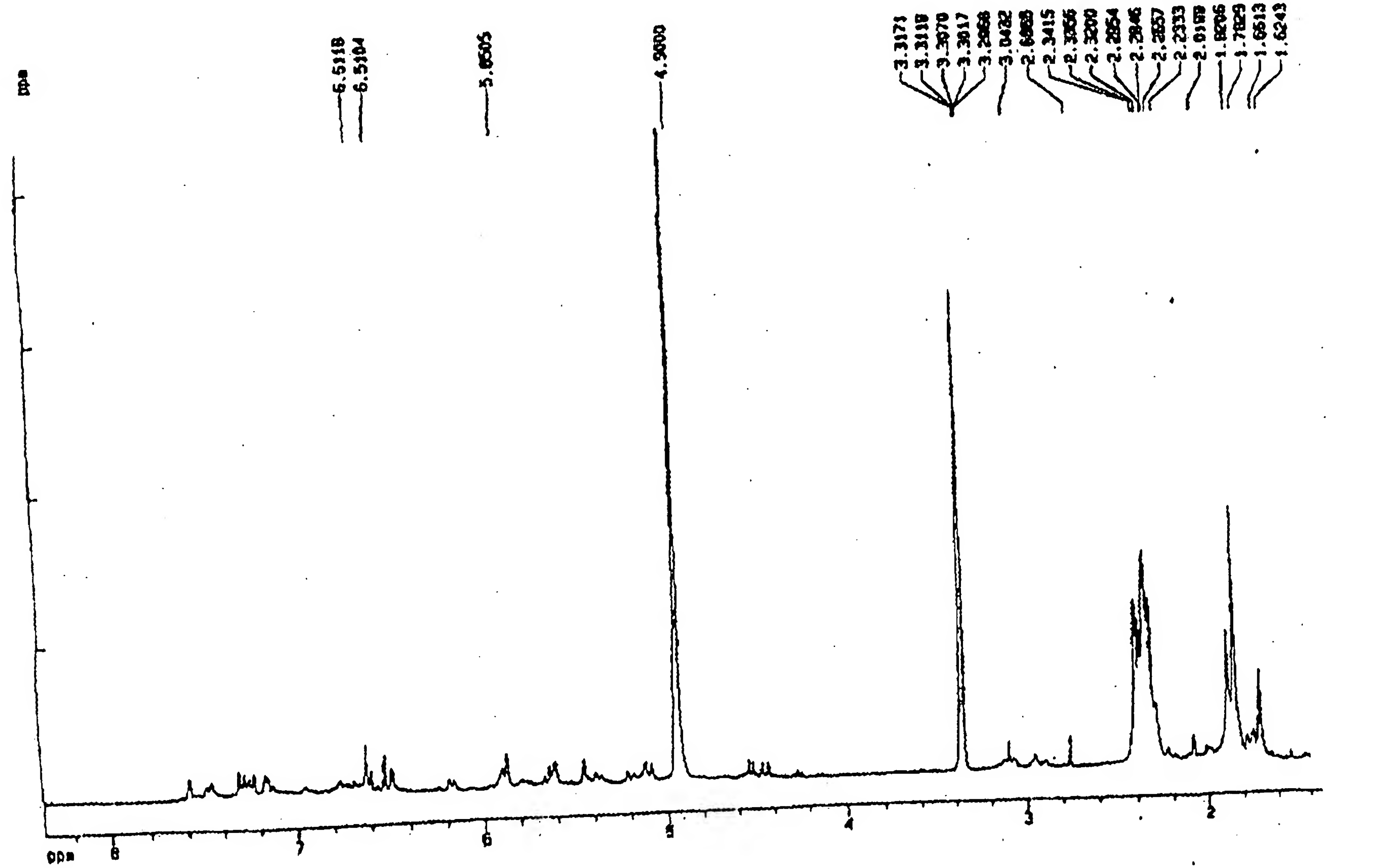
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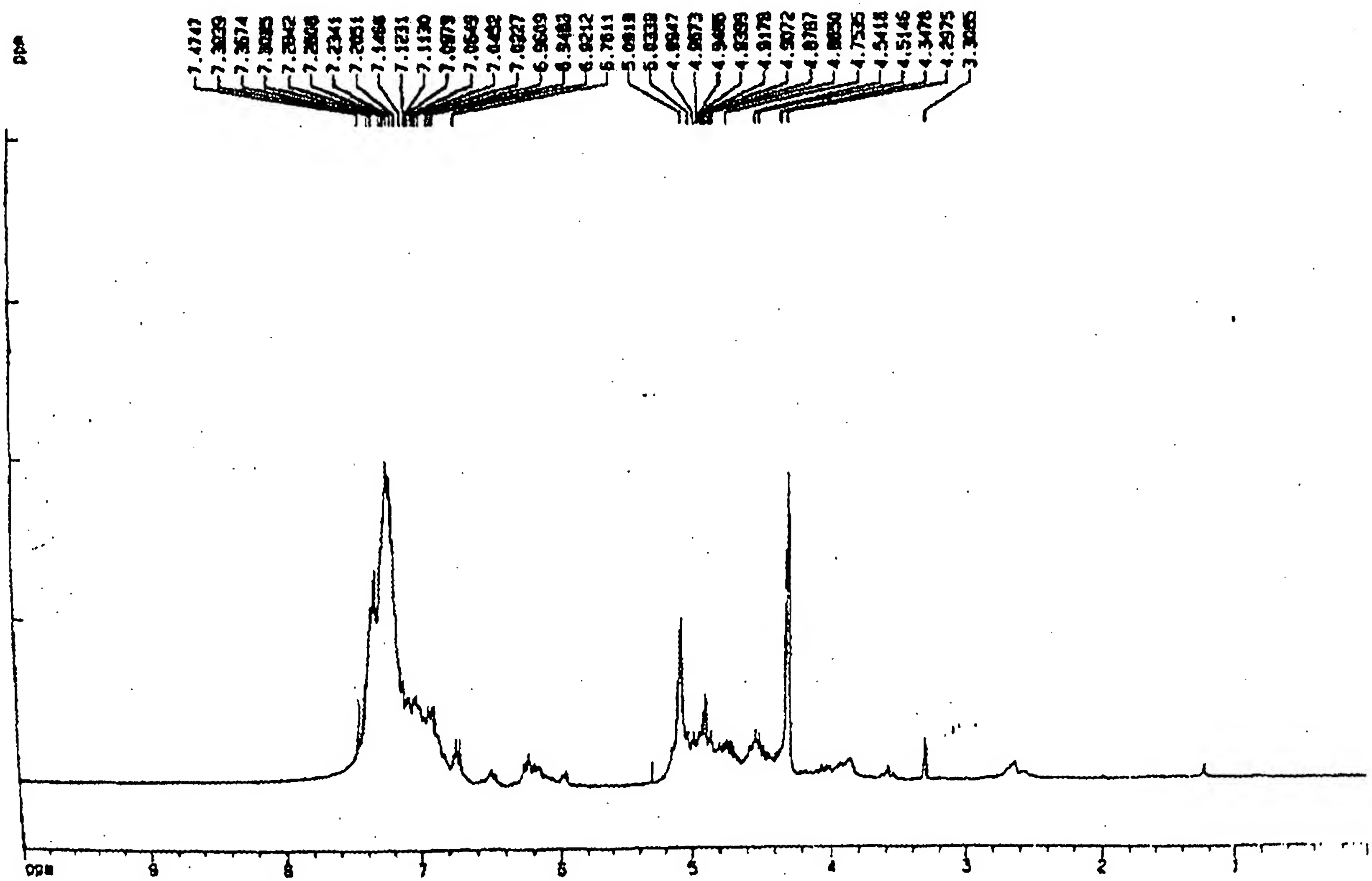
Figure 6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6.9609
6.9483
6.8212
6.7611
5.0918
5.0339
4.9947
4.9873
4.9466
4.9399
4.9178
4.9072
4.8787
4.8050
4.7535
4.5418
4.5146
4.3478
4.2975
3.3085
ppm
9
8
7
6
5
4
3
2
1

Absolute Structure Configuration for 8-bromo tetra-O-benzyl-(-)-epicatechin

Figure 7